

1 ATGGCGCCAC CACCAGCTAG AGTACATCTA GGTGCTGCTG TGGCACTGAC TGGCACTGAC GGGAGCGCAG CGAGTCGGAC AGAGGCAGCC GCGGCCACAC  
TACCGCGGTG GTGGTCGATC TCATGTAGAT CCACGCAAGG ACCGTACTG AGGCTTAGG CCTCGCGTC GCTCACCCCTG TCTCCGTCCG CCGCGGTGTG  
1 MetalaProp roProAlaAr gValHisLeu GlyAlaPheL euAlaValTh rProAsnPro GlySerAlaA laSerGlyTh rGluAlaAla AlaAlaThrPro

101 CCAGCAAAAGT GTGGGGCTCT TCCGCGGGGA GGATTGAACC ACAGAGCGGG GCGCGAGGAG CGCTCCCTAC CTCCATGGGA CAGCACGGAC CCAGTGCCCG  
GGTCGTTTCA CACCCCGAGA AGCGGCCCTT CCTNACTTGG TGCTCCGCCC CCGGCTCCTC GCGAGGGATG GAGGTACCTT GTCGTGCCCTG GGTACACGGC  
35 SerLysva lTrpGlySer SerAlaGlyA rgileGluPr oArgGlyGly GlyArgGlyA laLeuProTh rSerMetGly GlnHisGlyP roSerAlaArg

201 GGGCGGGCA GGGCGGGCC CAGGACCCAG GCGCGGGCGG GAAGCCAGCC CTCGGCTCCG GGTCCACAAG ACCTTCAAGT TTGTCGTCTG CCGGGTCTCTG  
CCGGGCCCGT CCGCGCGGGG GTCCCTGGGTG CCGCGCGGCC CTTCGGTCGG GAGCCGAGG CCAGGTGTTT TGGAAGTTCA AACAGCAGCA GCCCCAGGAC  
68 AlaArgAla GlyArgAlaP roGlyProAr gProAlaArg GluAlaSerP roArgLeuAr gValHisLys ThrPheLysP heValValva lGlyValLeu

301 CTGCAGGTG TACCTAGCTC AGCTGCAACC ATCAAACTTC ATGATCAATC AATTGGCACA CAGCAATGGG AACATAGCCC TTTGGGAGAG TTGTGTTCCAC  
GACGTCCAGC ATGGATCGAG TCGACGTTGG TAGTTTGAAG TACTAGTTAG TTAACCGTGT GTCGTTACCC TTGTATCGGG AAACCTCTC AACACAGGTG  
101 LeuGlnValv alProSerSe rAlaAlaThr lLeLysLeuH iAspGlnSe rileGlyThr GlnGlnTrpG luHisSerPr oLeuGlyGlu LeuCysProPr

401 CAGGATCTCA TAGATCAGAA CGTCTGGAG CCTGTAAACC GTGCACAGAG GGTGTGGGT ACACCAATGC TTCCAACAAT TTGTTTGTCT GCCTCCCATG  
GTCCTAGAT ATCTAGTCTT GCAGGACCTC GGACATTGGC CACGTGTCTC CCACACCCAA TGTGTTTACG AAGTTGTTA AACAAACGAA CCGAGGGTAC  
135 GlySerHi sArgSerGlu ArgProGlyA laCysAsnAr gCysThrGlu GlyValGlyT yrThrAsnAl aSerAsnAsn LeuPheAlaC ysLeuProCys

501 TACAGCTTGT AAATCAGATG AAGAAGAGAG AGTCCCTGC ACCACGACCA GGAACACAGC ATGTCAGTGC AAACCCAGAA CTTTCCGGAA TGACAAATTCT  
ATGTCGAACA TTAGTCTAC TTCTTCTCTC TTCAGGGACG TGGTGTGGT CCTGTGTGCG TACAGTACAG TTTGGTCTCT GAAAGGCCCTT ACTGTTAAGA  
168 ThrAlaCys LysSerAspG luGluGluAr gSerProCys ThrThrThra rAsnThral aCysGlnCys LysProGlyT hrPheArgas naSpasnsr

601 GCTGAGATGT GCCGGAAGTG CAGCACAGGG TGCCCCAGAG GGATGGTCAA GGTCRAAGAT TGTACGCCCT TGTACGCCCT GGAGTGACAT CGAGTGTGTC CACAAAGAAT  
CGACTCTACA CCGCCTTAC GTCGTGTCCC ACGGGGTCTC CCTACCAGTT CCAGTTCCCTA ACATGCGGGA CCTCACTGTA GCTCACACAG GTGTTTCTTA  
201 AlaGluMetC ysArgLysCy sSerThrGly CysProArgG lyMetVally svalLysasp CysThrProT rPserAspI lGluCysVal HisLysGlnSer

701 CAGGCAATGG ACATAATATA TGGGTGATT TGGTTGTGAC TTTGGTTGTT CCGTTGCTGT TGGTGGCTGT GCTGATTGTC TGTGTTGCA TCGGCTCAGG  
GTCCGTTACC TGTATTATAT ACCACTAAA ACCAACACTG AACCAACAA GGCAACGACA ACCACCGACA CGACTAACAG ACACCAACGT AGCCGAGTCC  
235 GlyAsnG lYHisAsnIle TrpValIleL euValValTh rLeuValVal ProLeuLeuL euValAlaVal lLeuIleVal CysCysCysI leGlySerGly

801 TTGTGGAGGG GACCCCAAGT GCATGGACAG GGTGTGTTTC TGGCGCTTGG GTCTCCTACG AGGGCCTGGG GCTGAGGACA ATGCTCACAA CGAGATTCTG  
AACACCTCCC CTGGGGTTCA CGTACCTGTC CCACACAAG ACCGGAACC CAGAGGATGC TCCCGGACCC CGACTCCTGT TACGAGTGT TGTCTAAGAC  
268 CysGlyGly AspProLysC ysMetAspAr gValCysPhe TrpArgLeuG lyLeuLeuAr gGlyProGly AlaGluAspA snAlaHisas nGluIleLeu

901 AGCAACGCAG ACTCGCTGTC CACTTTCGTC TCTGAGCAGC AAATGGAAG CCAGGAGCCG GCAGATTGA CAGGTGTAC TGTACAGTCC CCAGGGGAGG  
TCGTTGCGTC TGAGCGACAG GTGAAAGCAG AGACTCGTCG TTTACCTTTC GTCTCTGGC CGTCTAACT GTCCACAGTG ACATGTACAG GGTCCCTCC  
301 SerAsnAlaA spSerLeuse rThrPheVal SerGluGlnG lnMetGluse rGlnGluPro AlaAspLeut hrGlyValTh rValGlnSer ProGlyGluAla

1001 CACAGTCTCT CTTGGGACCG GCAGAAGCTG AAGGTCTCTA GAGGAGGAG CTGCTGGTTC CAGCAATGG TGCTGACCCC ACTGAGACTC TGATGCTGT  
GTGTACAGA CGACCTGGC CGTCTTCGAC TTCCACAGAT CTCCTCCTCC GACGACCAAG GTCGTTTACC ACGACTGGG TGACTCTGAG ACTACGACAA  
335 GlnCysLe uLeuGlyPro AlaGluAlag luGlySerG lNargArgArg LeuLeuValP roAlaAsnG lYalaAspPro ThrGluThrL euMetLeuphe

Fig. 1

1101 CTTTGACAAG TTTGCAAAACA TGTGTGCTCTT TCACTTCTCTT GATGAGCTCA ATGAGGAACTT GACCTCACC CAGAGCTGGT CAGAGCTGGT  
368 PheAspLys PheAlaAsnI leValProPh eAspMetTrp AspGlnLeuM etArgGlnLe uAspLeuThr lysAsnGlnI leAspValVa lArgAlaGly  
1201 ACAGCAGGCC CAGGGGATGC CTTGTATCCA ATGCTGATGA AATGGGTCAA CAAAACCTGGA CGGAACGCCT CGATCCACAC CCTGCTGGAT GCCTTGGAGA  
401 ThrAlaGlyp roGlyAspAl aLeuTyrAla MetLeuMetL yStrpValas nLysThrGly ArgAsnAlas erIleHisTh rLeuLeuAsp AlaLeuGluArg  
1301 GGATGGAAGA GAGACATGCA AAAGAGAAGA TTCAGGACCT CTTGGTGGAC TCTGAAAGT TCATCTACTT AGAAGATGGC ACAGGCTCTG CCGTGTCTT  
435 MetGluG1 uArgHisAla LysGluLysI leGlnAspLe uLeuValasp SerGlyLysP heIleTyrLe uGluAspGly ThrGlySera lavalSerLeu  
1401 GGAGTGA  
CCTCACT  
468 GluOP\*

Fig. 1 (cont.)

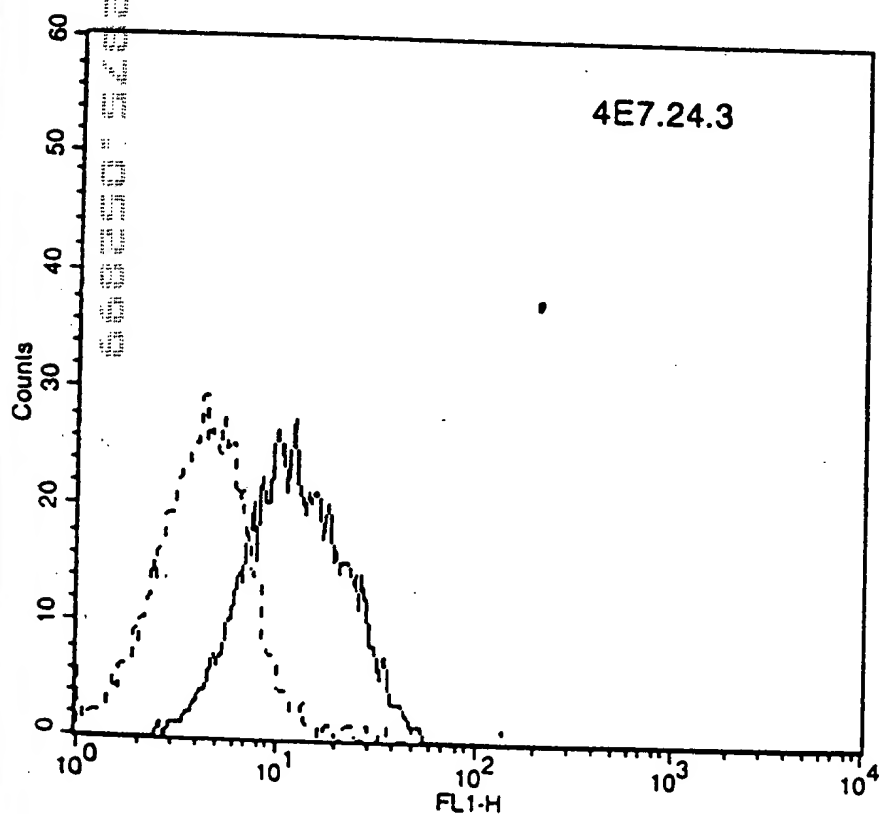
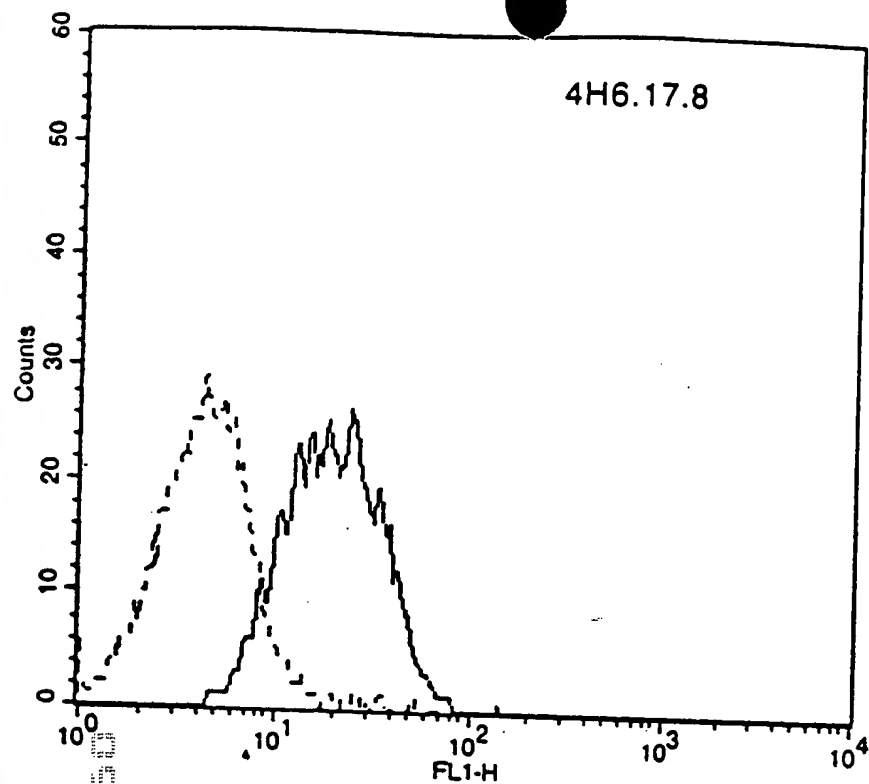


Fig. 2

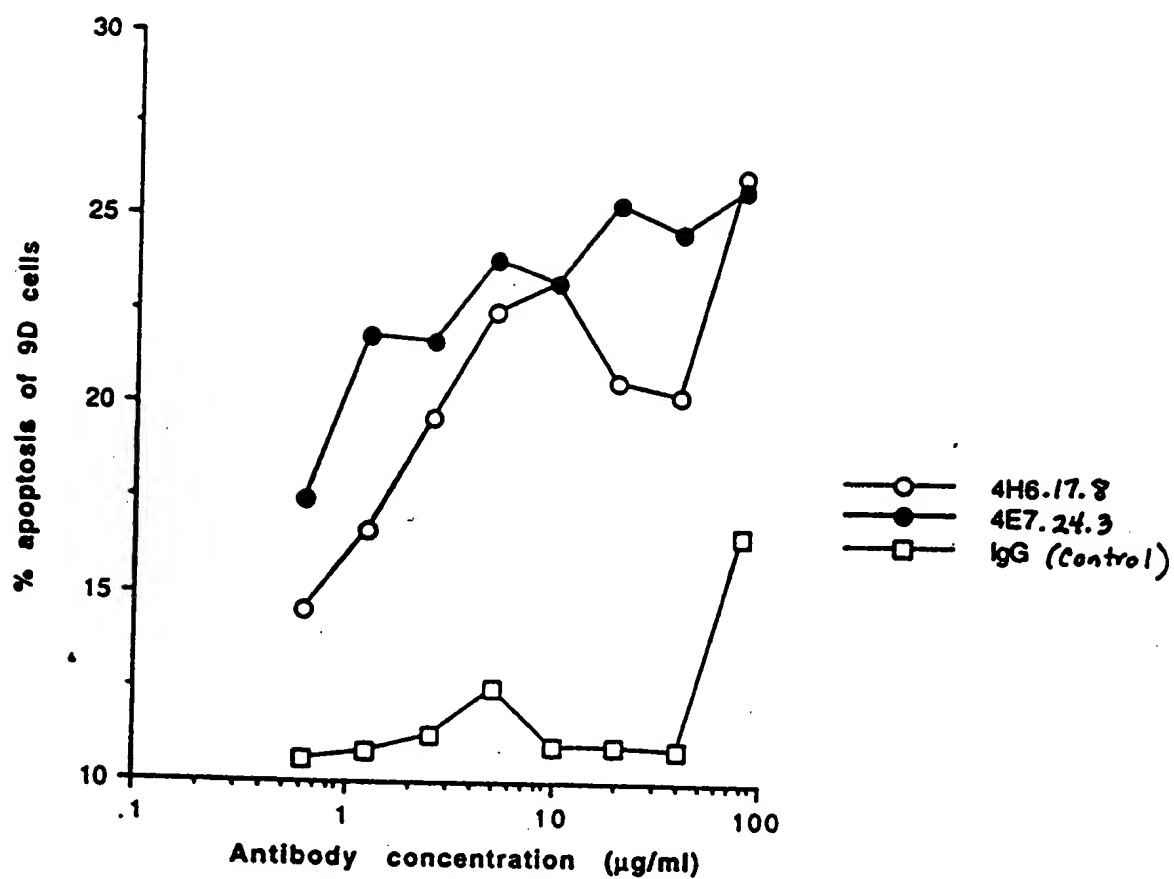


Fig. 3

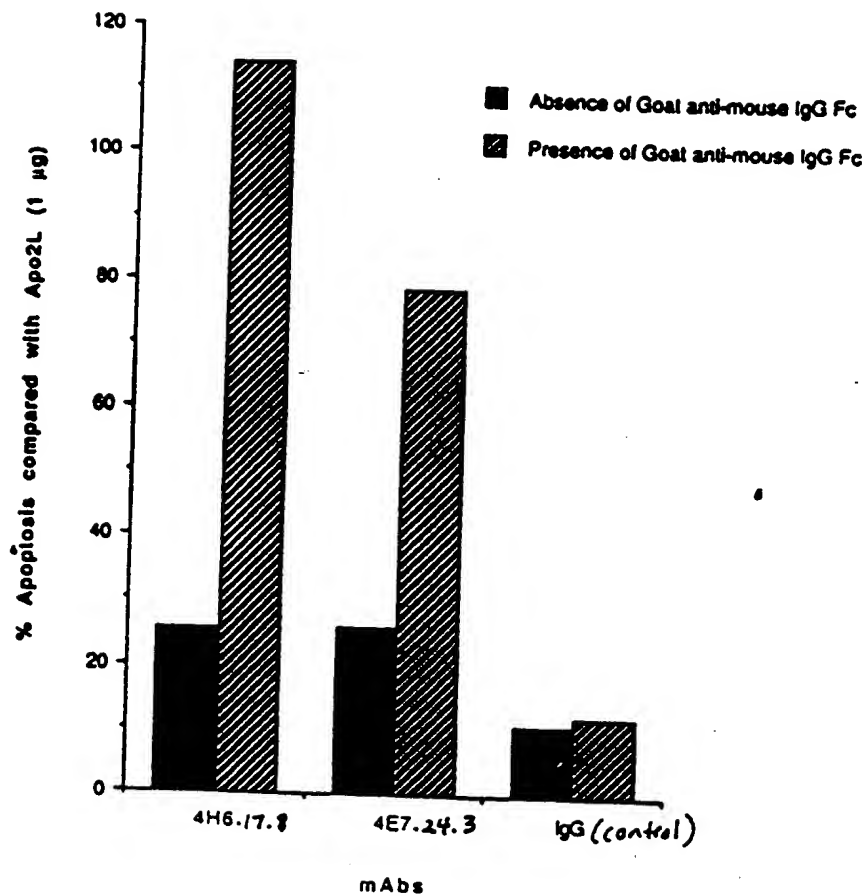


Fig. 4

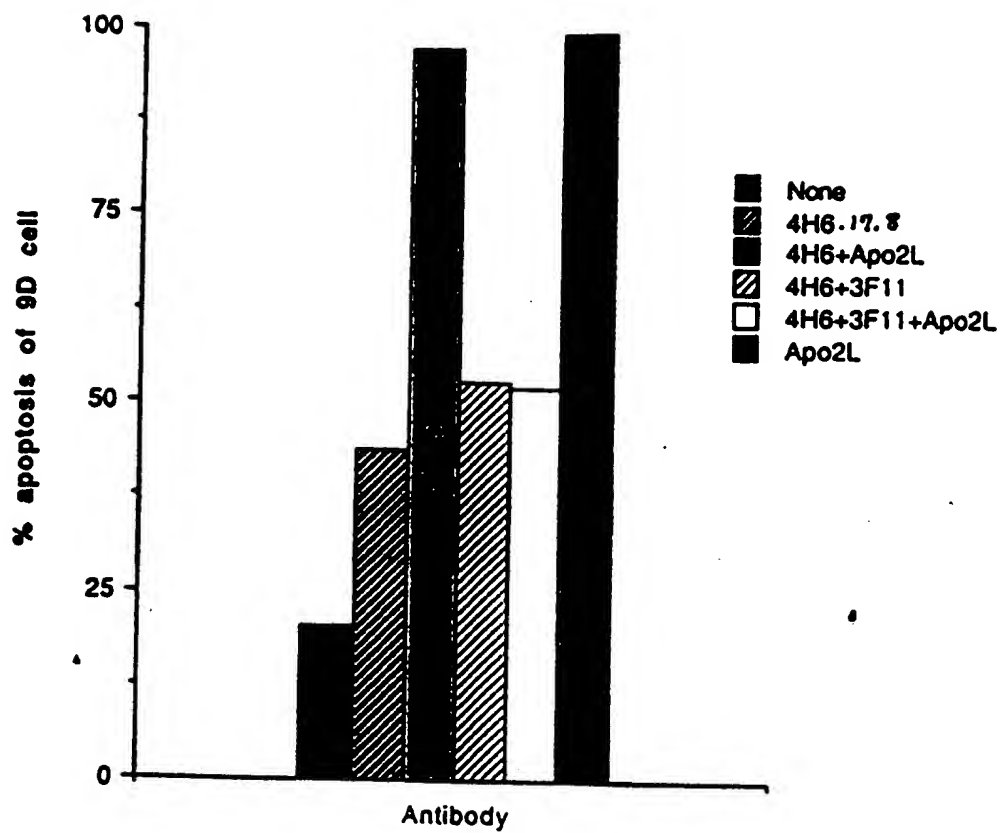
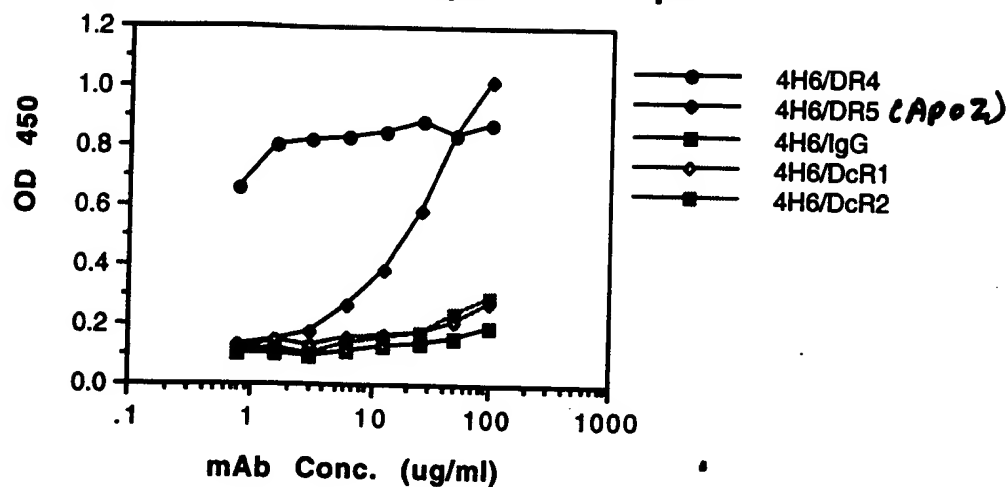


Fig. 5

#### 4H6: Binding to receptors for Apo2L



#### 4E7: Binding to Receptors for Apo2L

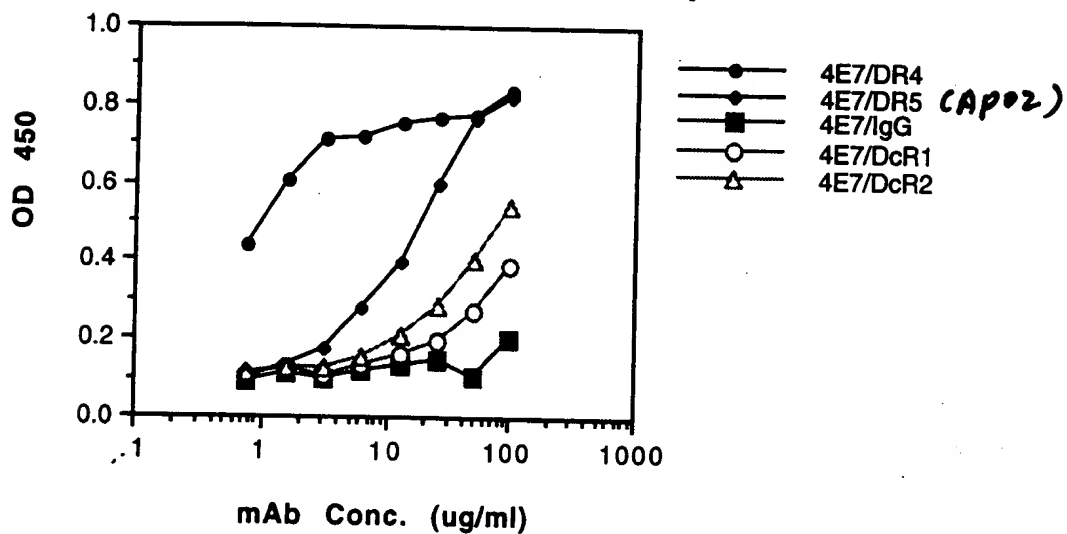


Fig. 6

## Affinities of Apo2Rs and mAbs

		Affinity (pM)
DR4-IgG	to Apo2L	82
DR5-IgG	to Apo2L	1
mAb 4E7	to DR4-IgG	2
mAb 4H6	to “	5
mAb 5G11	to “	22
mAb 3F11	to DR5-IgG	20
mAb 3H3	to “	3

Affinities were determined using KinExA

Fig. 7



# Apoptosis of 9D cells by anti-DR4 mAb plus complements or goat anti-mouse Ig-FC

Percent Apoptosis compared to Apo2L

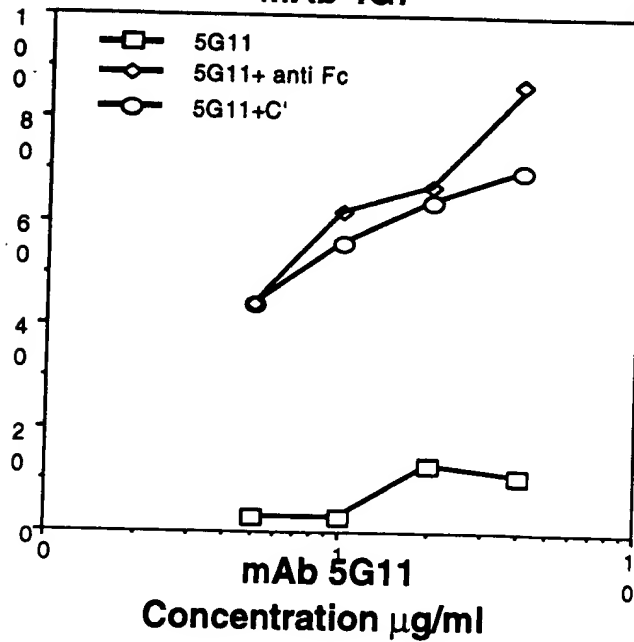
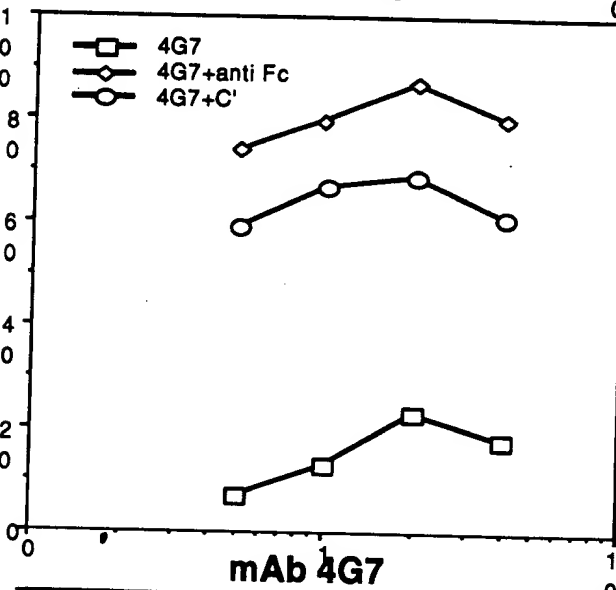
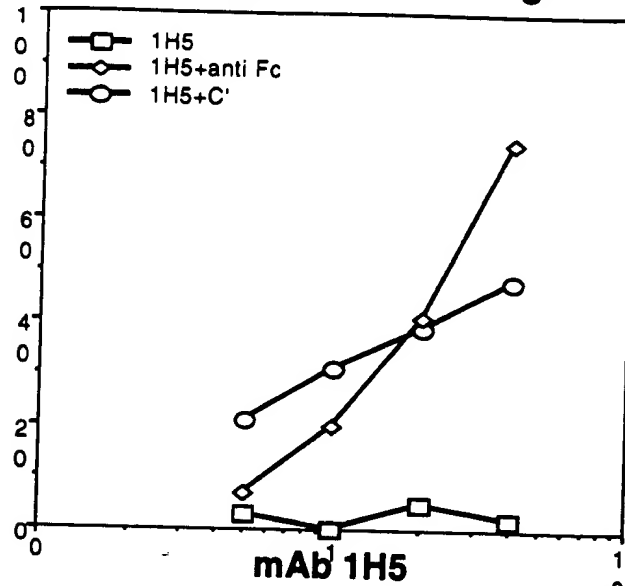


Fig. 8A

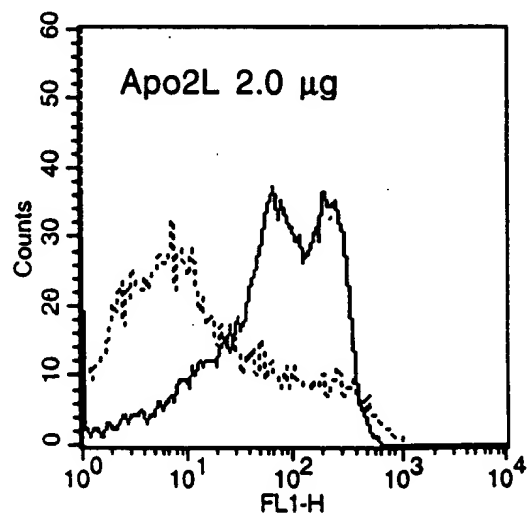
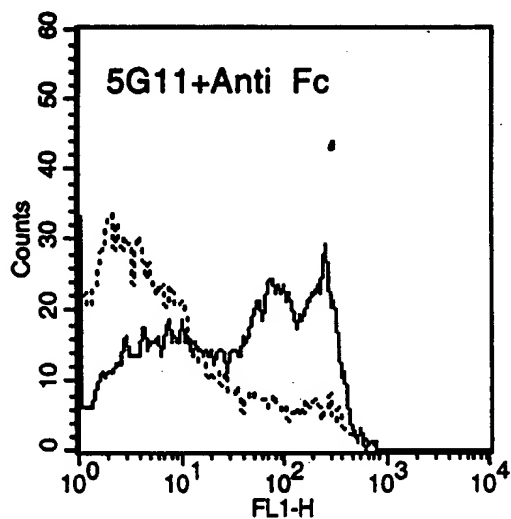
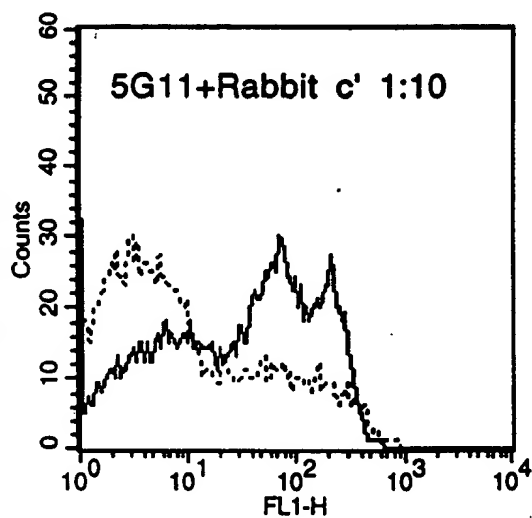
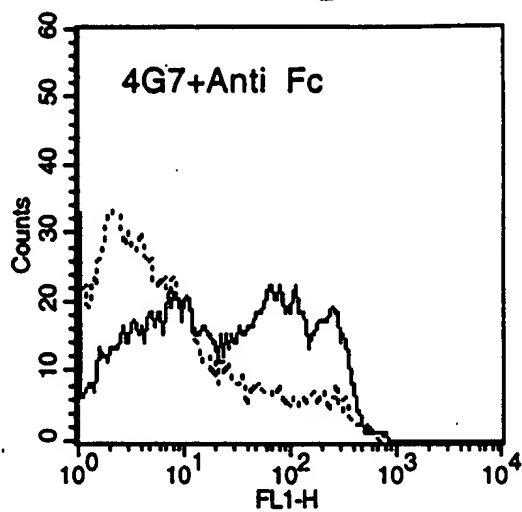
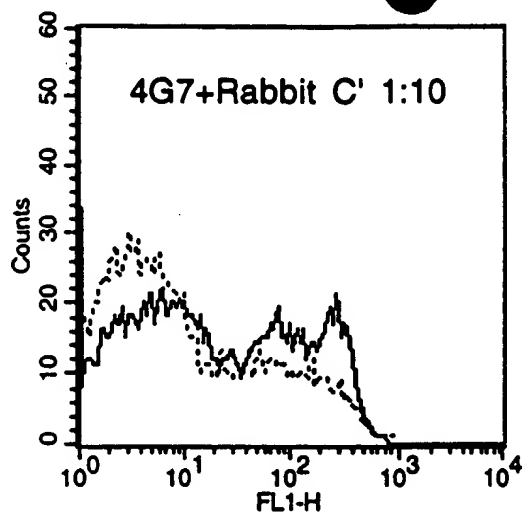


Fig. 8B

Fig. 9

plate 8-DR4 Mab

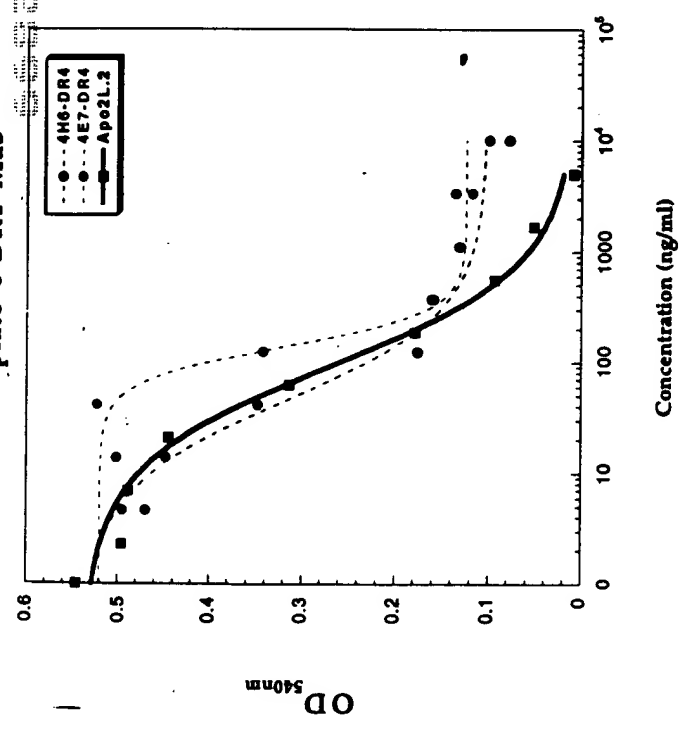


plate 9-DR4 Mab

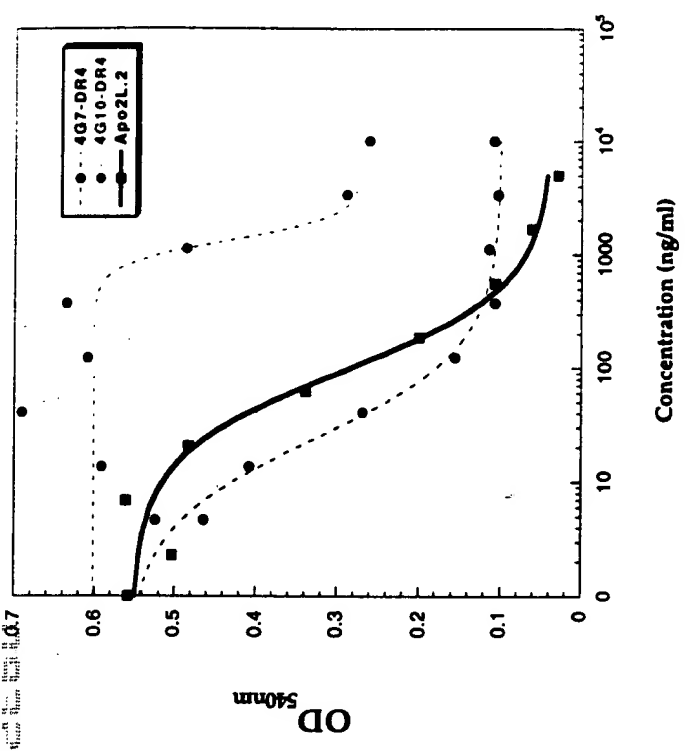


plate 7-DR4 Mab

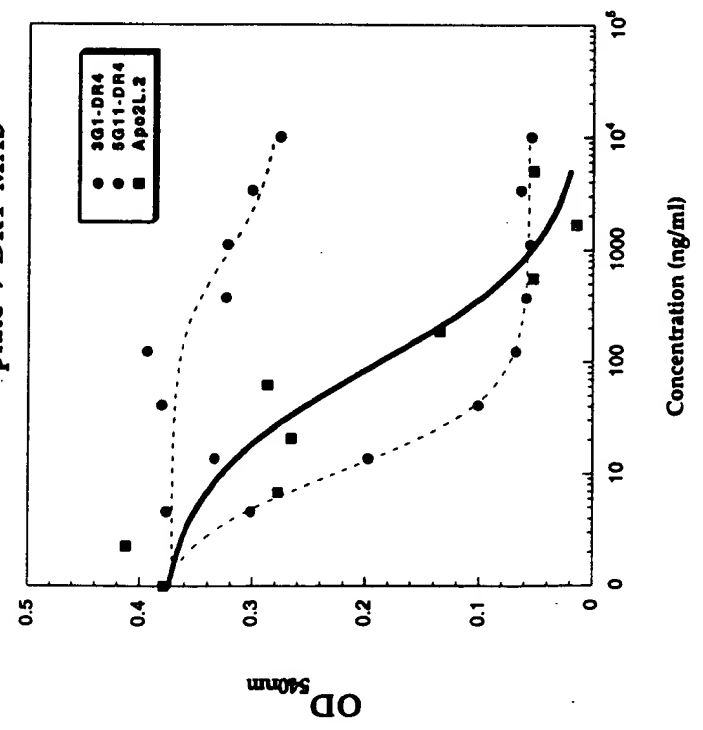
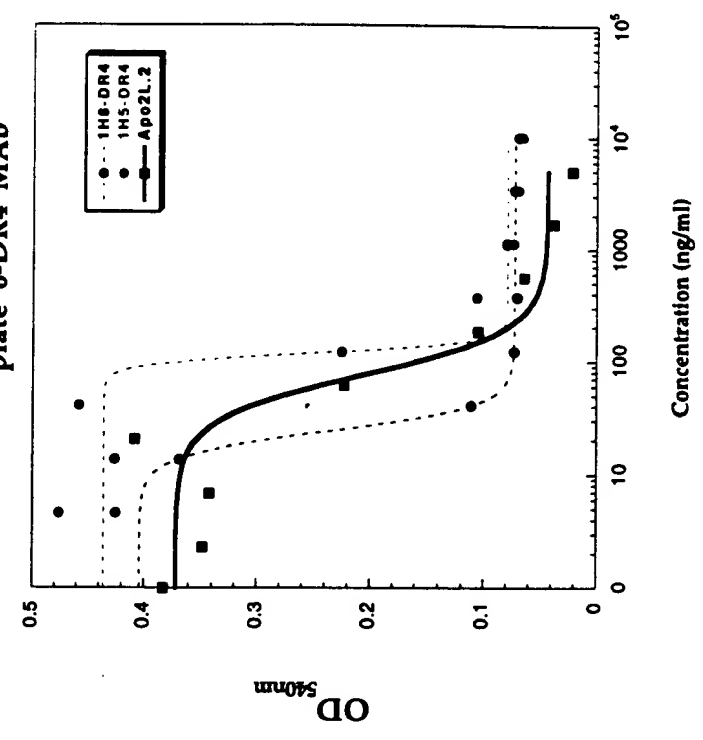


plate 6-DR4 Mab



## Apoptosis of anti DR4 mAbs plus goat anti FcAb

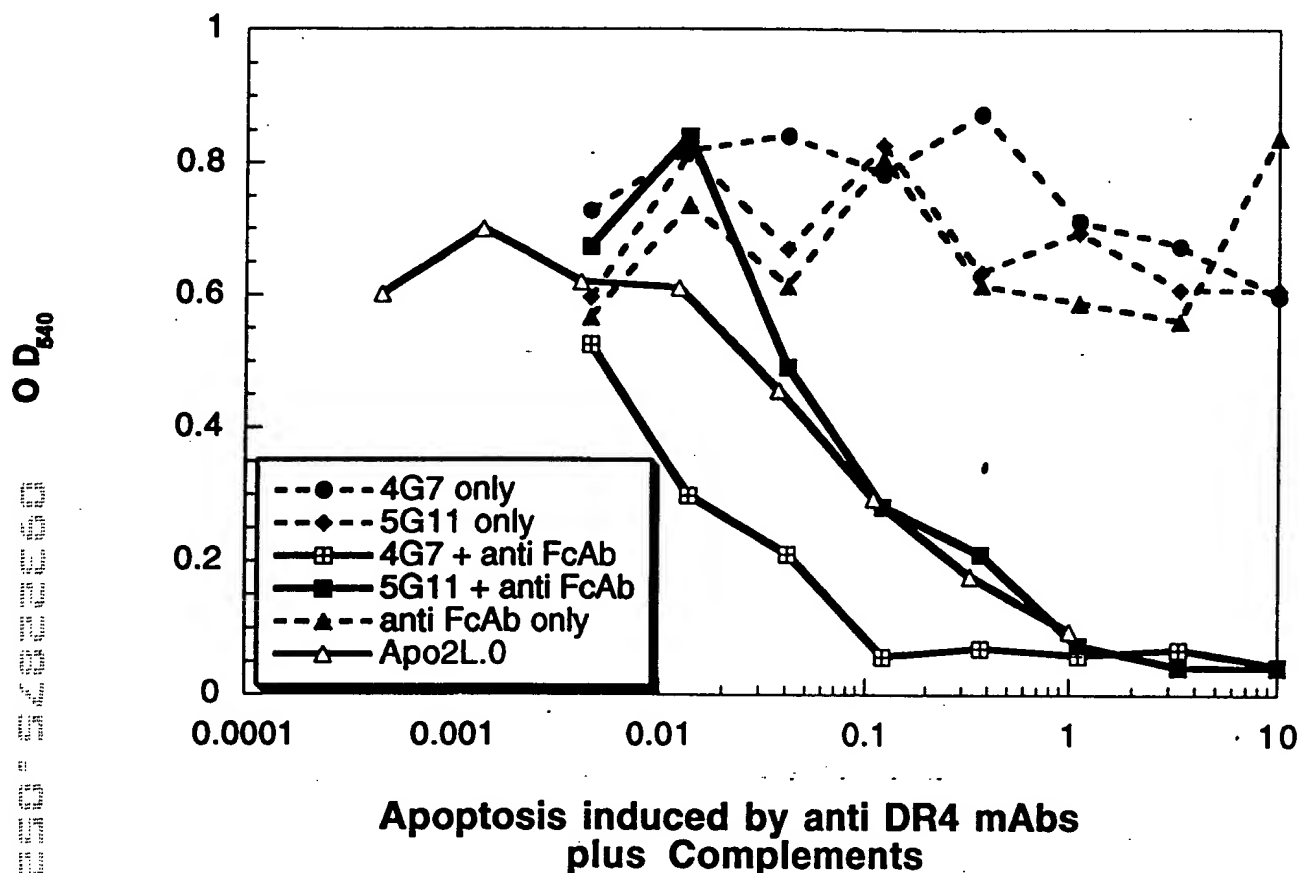


Fig.10A

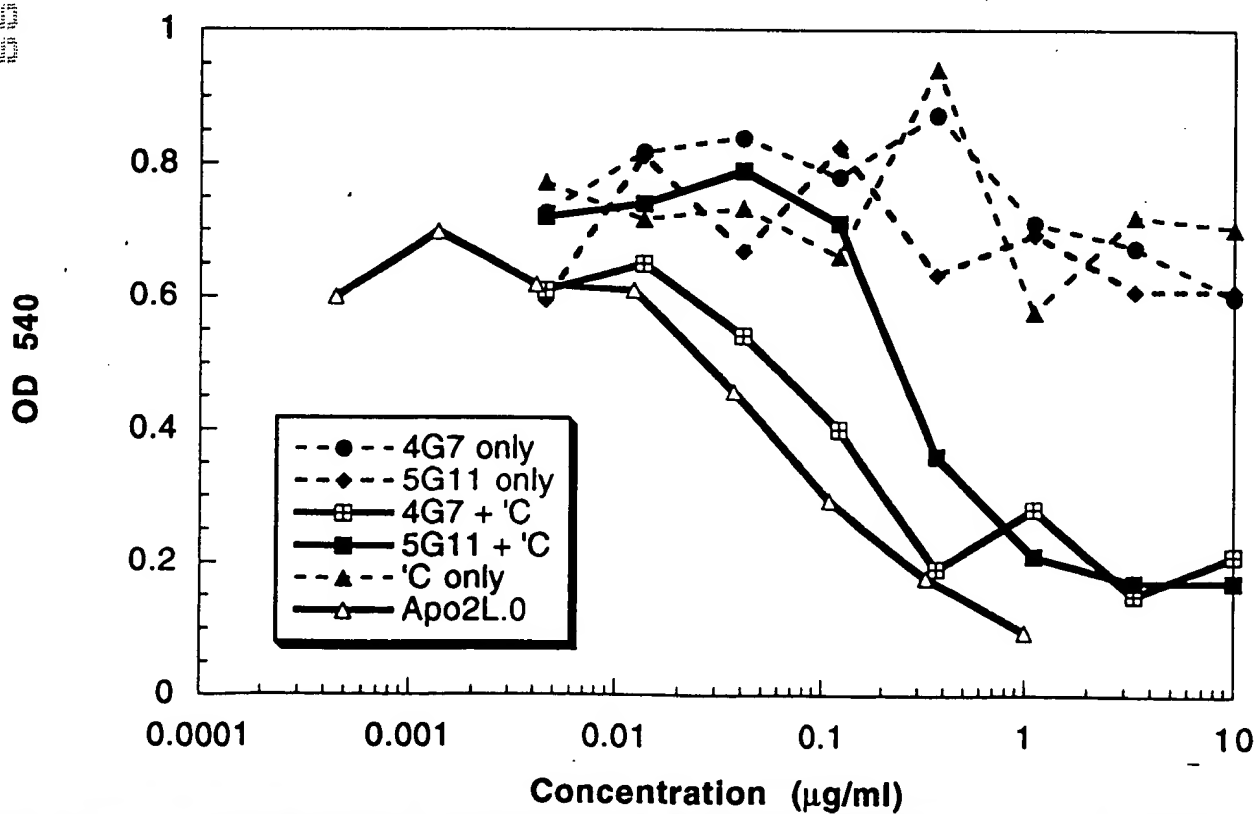
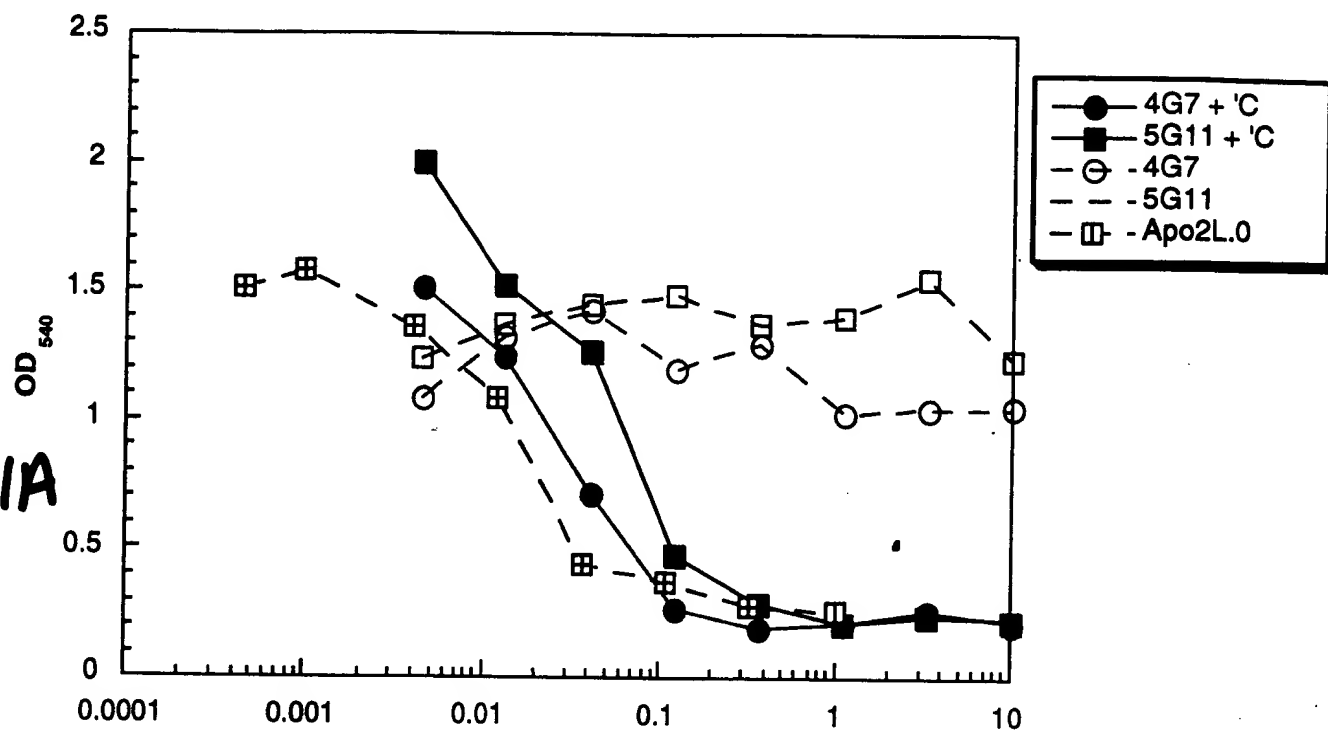
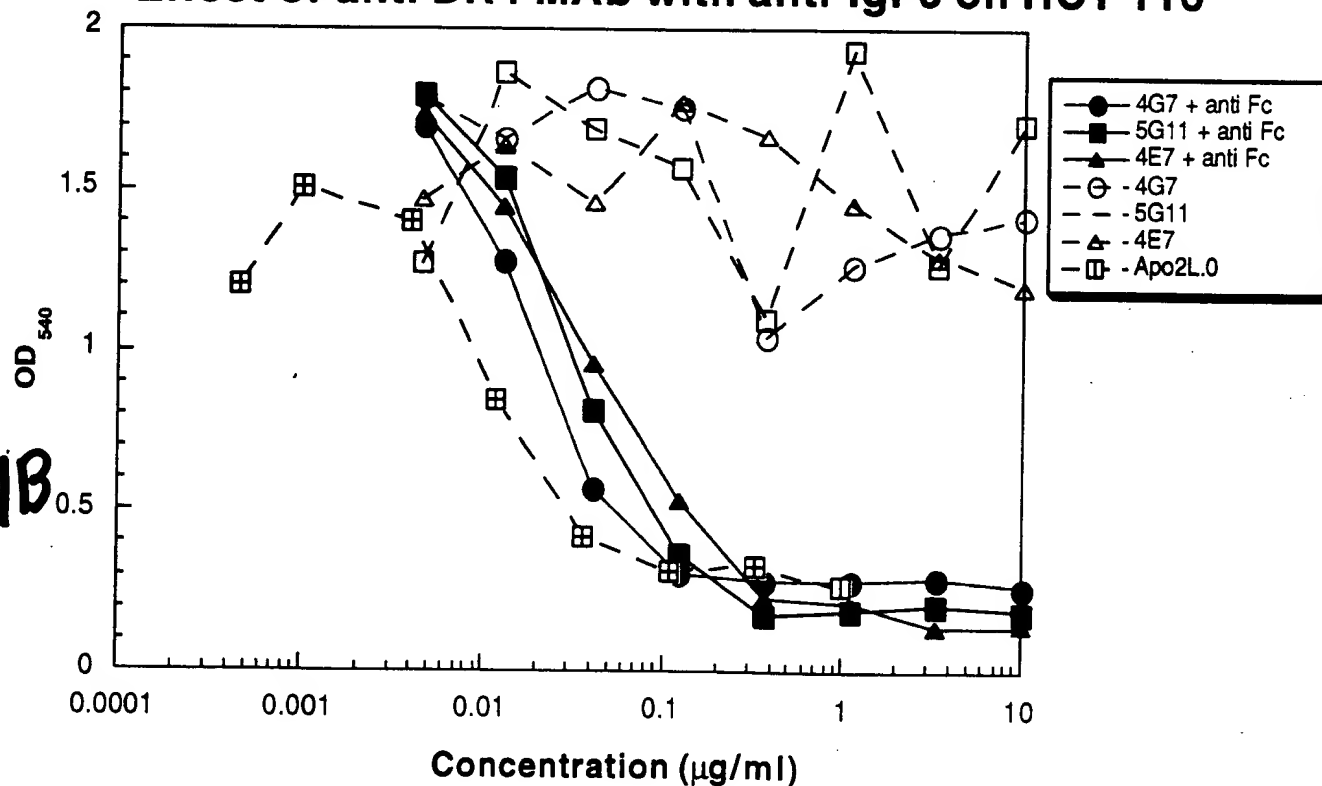


Fig.10B

## Effect of anti DR4 MAb with complement on HCT 116



## Effect of anti DR4 MAb with anti-IgFc on HCT 116



45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

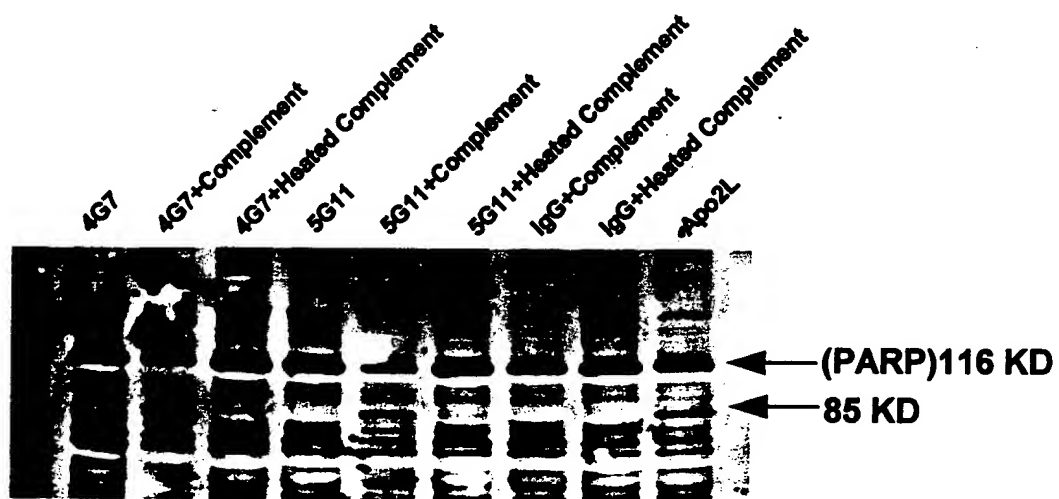


Fig. 12

5

Fig. 13

Effect of anti-DR4 mAbs  
on the HCT-116 tumor Weight on Day 50

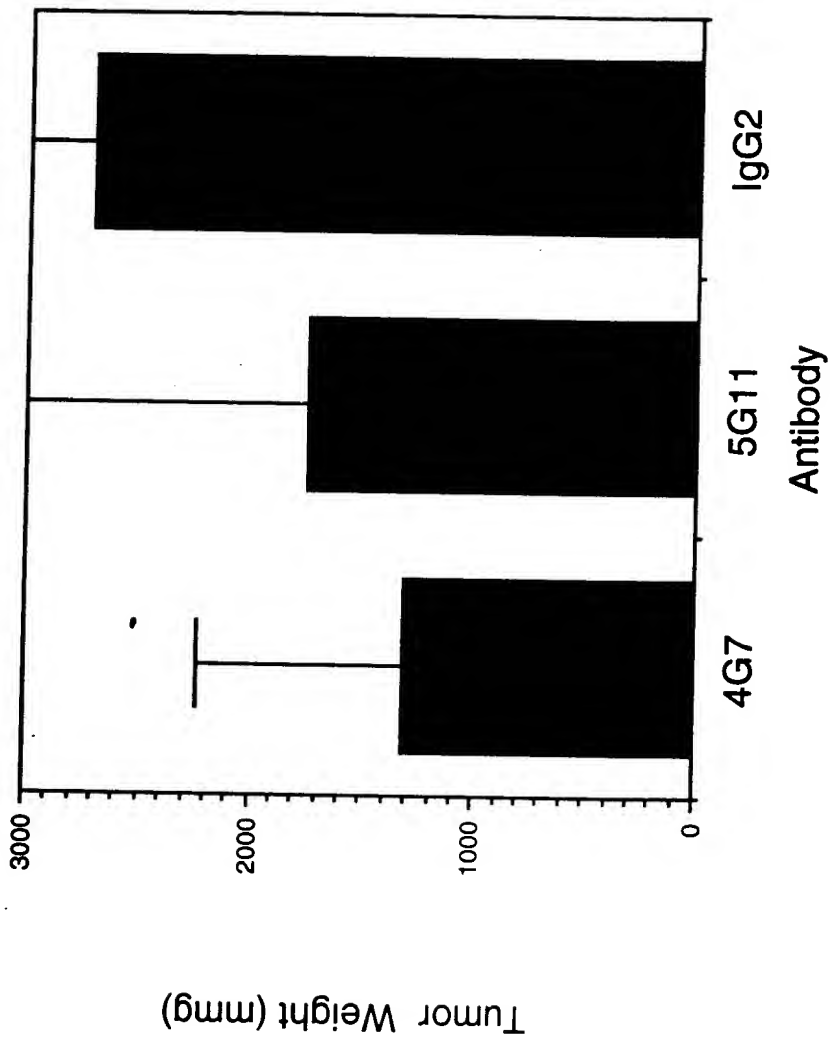
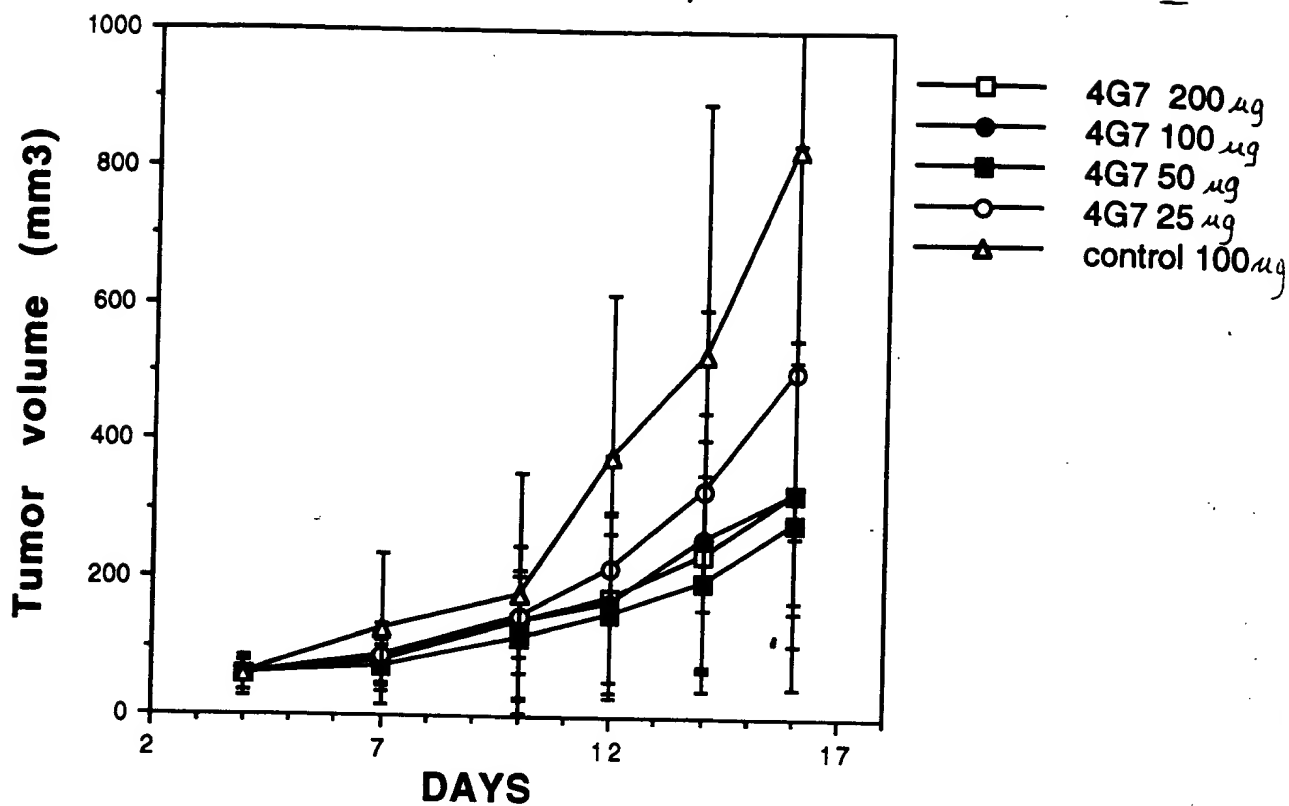


Fig. 14



Colo 205 (98-296 D)



Colo 205 (98-296C)

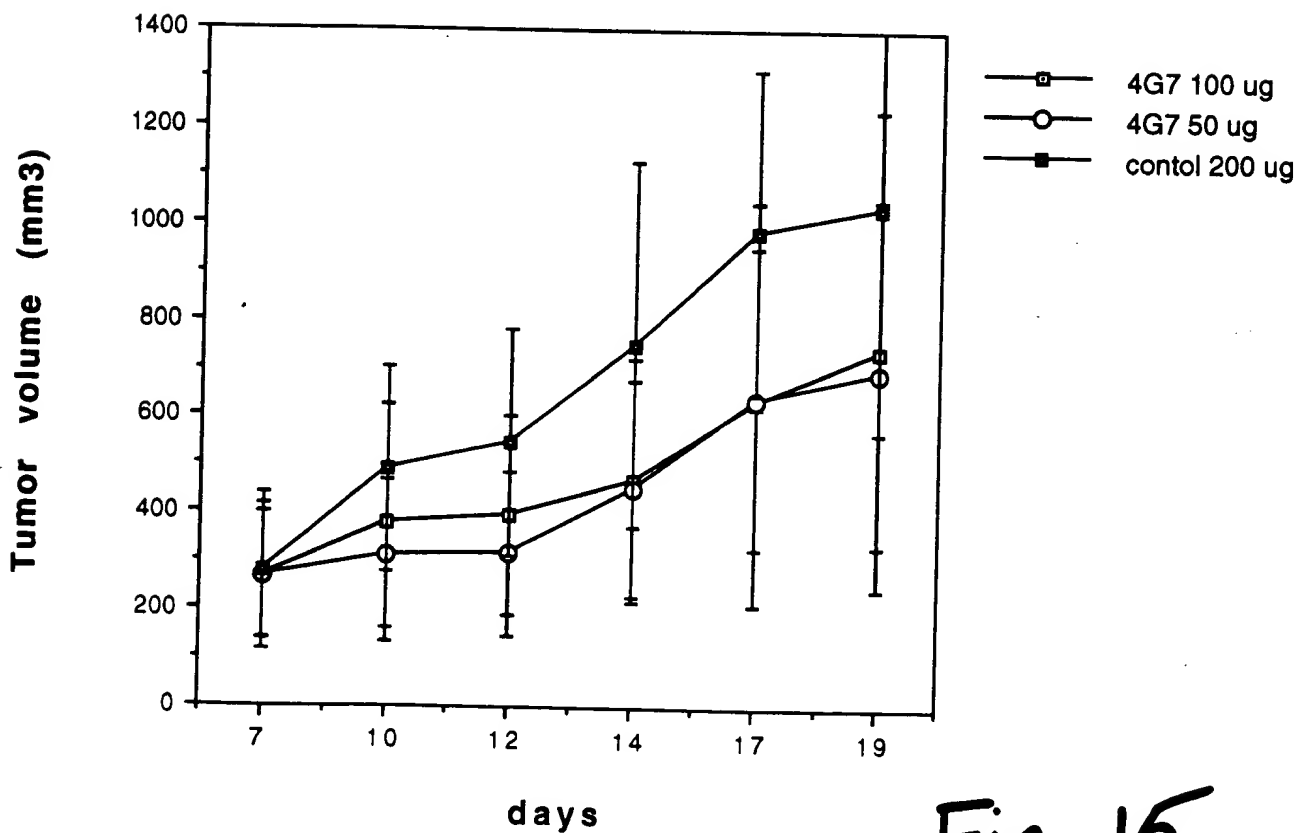


Fig. 15

**Colo 205 ( 98-296 D)**

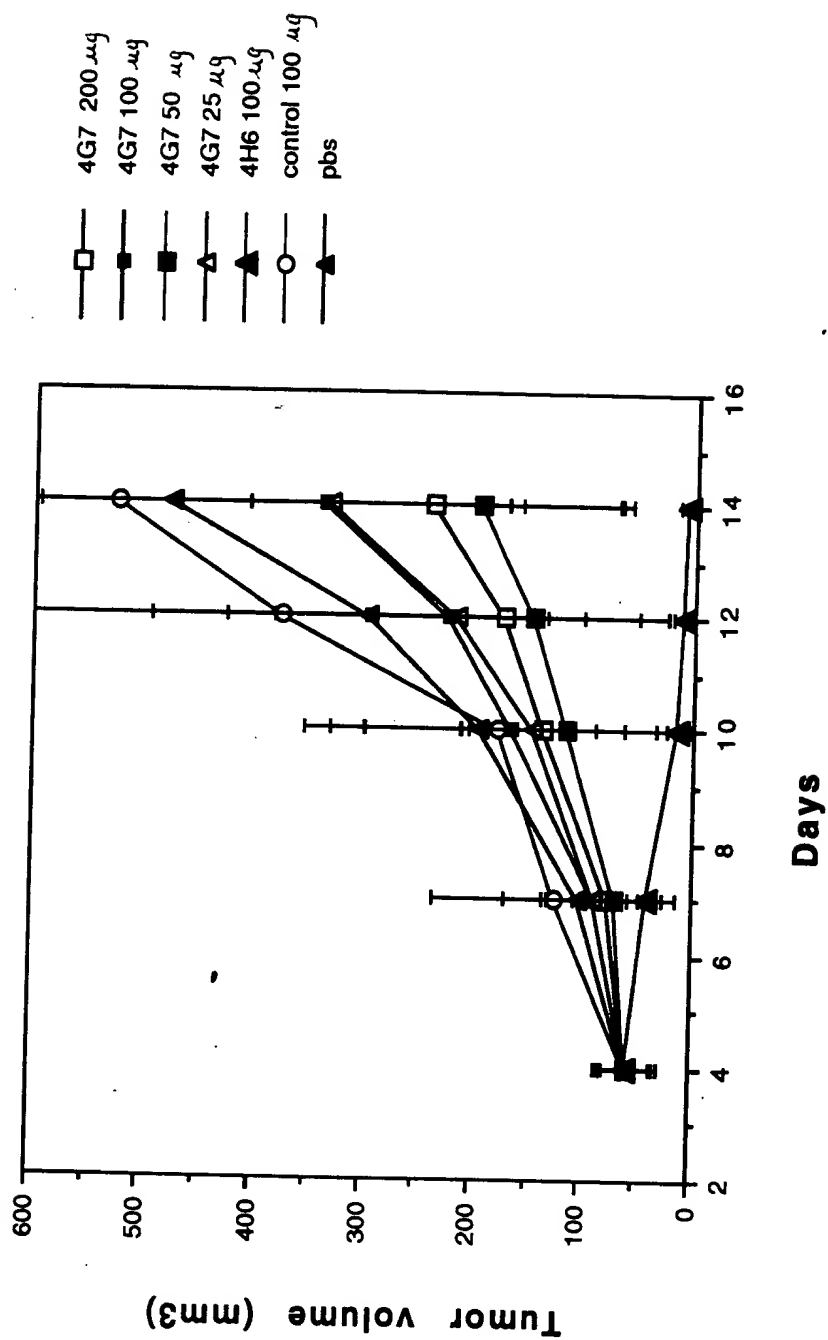


Fig. 16

## General Characteristics of Anti-DR4 mAbs

	Isotype	Kd-1 (pM)	Apopt w/o L	Apopt + &FC	Apopt + C'	Block	Cross reactivity			
							DR4	DR5	DCR1	DCR2
1H5.24.9	IgG2a		-	-	-	ND	+++	-	-	-
1H8.17.5	IgG1		+	ND	ND	ND	+++	-	-	-
3G1.17.2	IgG1		-	ND	ND	-	+++	-	-	-
4E7.24.3	IgG1	2	+	-	-	-	+++	+	-	+/-
4G7.18.8	IgG2a		+	+	+	-	+++	-	-	-
4H6.17.8	IgG1	5	+	-	-	+	+++	+	-	-
4G10.20.6	IgG1		+	ND	ND	-	+++	+	-	-
5G11.17.1	IgG2b	22	+	+	+	ND	+++	++	-	-

All these mAbs recognize DR4 on 9D cells and immune precipitate DR4-IgG.

w/o L: The apoptotic ability of mAbs by themselves was detected on 9D cells, skmes cells, HCT116 and colo 205

+ &FC: The apoptotic ability of mAbs was determined in combination with goat anti-mouse IgG FC.

+ C': The apoptotic ability of mAbs was determined in the presence of rabbit complement

Degrees of binding (+) to DR5 by Mabs 4E7 and 4H6 at 10 ug/ml are 15% of the binding to DR4.

Fig . 17